REMARKS

Claims 1-9 and 11-21 are pending. New claim 30 has been added. Support for new claim 30 is found in the specification and claims as originally filed, including, in original claim 3.

Clarification from the Examiner is requested as to the status of claim 19. In particular, in paragraph 5, the Examiner indicates that claim 19 is withdrawn from further consideration. However, claim 19 has been included in the rejections set forth in paragraphs 11, 12, 14 and 16.

The specification has been amended to update the cross-reference to related applications section, to correct a minor typographical error, and to capitalize the trademarks QIAQUICK, MIRACLOTH and SORVALL, as requested by the Examiner in paragraphs 7, 8 and 9.

Claims 1, 3, 4, 7, 9, 11, 12, 13, 17 and 18 have been amended as follows:

Claim 1 has been amended to clarify what is meant by the term "variant." Support for this amendment is found through out the specification and claims as originally filed, including at page 5, line 8 to page 6, line 32. Claim 1 has also been amended to refer to the fungal selection marker sequence as a "fungal selection marker polynucleotide sequence" and to refer to the fungal replication initiating polynucleotide sequence as a "fungal replication initiating polynucleotide sequence," in order to refer to these sequence in the dependent claims without creating confusion as to which sequence Applicant is referencing.

Claim 3 has been amended to recite that the polynucleotide sequence of interest further comprises a control sequence.

Claim 4 has been amended to depend from claim 1 instead of claim 3 and to recite "wherein the polynucleotide sequence of interest encodes a hormone, an enzyme, a receptor or a portion thereof, an antibody or a portion thereof, or a reporter, or a regulatory protein."

Claim 7 has been amended to depend from claim 3, instead of claim 1, so as to provide proper antecedent basis for "the control sequence."

Claim 9 has been amended to recite "the selection marker polynucleotide sequence," in order to provide proper antecedent basis. Claim 9 has also been amended to recite that the selection maker polynucleotide sequence is selected from a group of genes which encode a product "which is responsible" for one of a list or functions/activities, as suggested by the Examiner.

Claim 11 has been amended to provide proper antecedent basis.

Claim 12 has been amended to change the identity from 50% to 80%. Support for this amendment is found in the specification on page 13, line 1. Claim 12 has also been amended to delete

phrase (c). Further, claim 12 has been amended to include a reference to the GAP computer program used for determining identity of sequences, as described in the specification on page 13.

Claim 13 has also been amended to include a reference to the GAP computer program, used for determining identity of sequences. Claim 13 has also been amended to provide proper antecedent basis and clarification of the term "nucleic acid sequence."

Claim 14 has been amended to provide proper antecedent basis.

Claim 17 has been amended to delete the phrase "or is a respective functional subsequence thereof." Claim 17 has also been amended to provide proper antecedent basis.

Claim 18 has been amended to provide proper antecedent basis and to remove the term "preferably."

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Written Description Rejection of Claims 1-9, 11-21 under 35 U.S.C. 112 for

Claims 1-9 and 11-21 stand rejected under 35 U.S.C. 112, first paragraph, as allegedly not complying with the written description requirement of the Patent Code. The Examiner contends that the specification does not adequately provide written description support for the phrases "a replication initiating sequence having at least 50% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or its respective functional subsequence thereof' (claims 12-16), "a portion of receptor" (claim 4), and "a portion of an antibody" (claim 4).

Initially, Applicant submits that claims 1-9, 11 and 18-21 have been improperly included in the pending written description rejection because these claims do not include any of the terms or phrases that the Examiner asserts lack adequate written description support. Thus, Applicant requests reconsideration and withdrawal of the rejection as applied to claims 1-9, 11 and 18-21.

As to claims 12 and 13, which include the recitation "having at least 50% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2," Applicant respectfully disagrees with the Examiner's conclusion that the specification does not sufficiently describe replicating initiating sequences having at least 50% identity with SEQ ID NOS. 1 and 2. However, in order to expedite prosecution, claims 12 and 13 have been amended to specify that the degree of identity is at least 80%. Support for this amendment is found in the specification on page 13, line 1. In this regard, the specification clearly provides written description for sequences having this very high degree of identity

to SEQ ID NOS. 1 and 2, as this very high degree of identity provides a clear structural description of the nucleic acid sequences at issue. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection in view of these amendments.

The Examiner also contends that the recitations "a receptor or a portion thereof" and "an antibody or a portion thereof," appearing in claim 4, lack adequate written description because the specification does not sufficiently teach "that the portion of the polypeptide would exhibit the same or similar property as the encoded proteins." Applicant respectfully submits that the assertion of a written description rejection based on the use of these phrases is clearly not appropriate. Foremost, Applicant is not claiming receptors or antibodies per se (or receptors or antibodies that have a specific function or activity), but rather Applicant's invention provides methods for constructing and screening or selecting libraries of polynucleotides sequences that can be applied to any polypeptide. As disclosed in the specification, the methods of the present invention are applicable to all types of sequences encoding polypeptides, including full polypeptides and to portions of polypeptides. See the specification at page 7, line 13 to page 8, line18. It is simply not required for the methods of the present invention that the receptor or antibody exhibit the same or similar properties of the full receptor or antibody or that a specific sequence of the receptor or antibody sequence be included in "the portion." Accordingly, Applicant respectfully requests reconsideration and withdrawal of the 112 rejection.

II. The Enablement Rejection of Claims 1-9, 11-21 under 35 U.S.C. 112

Claims 1-9 and 11-21 stand rejected under 35 U.S.C. 112, first paragraph as allegedly non-enabled. In particular, the Examiner alleges that although the specification is enabled for replicating initiating sequences set forth in SEQ ID NO:1 or SEQ ID NO:2, that the specification does not reasonably provide enablement for replication initiating sequence with at least 50% identity to SEQ ID NO:1 or SEQ ID NO:2. The Examiner further contends that since the nucleic acid sequence of a polynucleotide determines its protein coding properties, predictability of which changes can be tolerated requires a knowledge of, and guidance with regard to which nucleic acids in the nucleotide sequences, if any, are tolerant of modification and which are conserved (i.e., unexpectedly intolerant to modification), and detailed knowledge of the ways in which the product's structure relates to its usefulness. This rejection is respectfully traversed.

First, Applicant notes that the replication initiating sequences likely do not contain open reading frames that encode polypeptides. Thus, the Examiner's concerns about the predictability of the protein coding properties due to changes to the nucleic acid sequence are likely not applicable.

Furthermore, the phrase "having at least 50% identity to SEQ ID NO:1 or SEQ ID NO:2," as used in dependent claims 12 and 23, merely recites, in view of Applicant's results with SEQ ID NO:1 and SEQ ID NO:2, sequences which an artisan can also reasonably expect, due to the high degree of sequence identity to SEQ ID NO's 1 and 2, to function well in the methods of the present invention. An artisan would clearly be able to obtain such sequences without routine experimentation. Nevertheless, in order to expedite prosecution, the claims have been amended to specify a degree of identity of at least 80%. Support for this amendment is found in the specification on page 13, line 1. It is clearly well within the skill of the artisan to make or obtain sequences having at least 80% identity to SEQ ID NOS. 1 or 2, which function as replication initiating sequences.

Finally, although the enablement rejection should now be withdrawn in view of the amendment, Applicant also respectfully submits that claims 1-9, 11, 18 and 20-21 were not properly included in the enablement rejection for the reasons of record because these claims do not include the recitation alleged to lack enablement. However, to the extent that the Examiner is rejecting claims 1-9, 11, 18 and 20-21 for lack enablement because they allegedly encompass inoperative embodiments recited by the recitations in claim 12 or 13, Applicant also submits that this rejection is still not appropriate. Indeed, it is well established that claims need not specifically exclude possible inoperative embodiments in order to be enabled, unless the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention. *See Atlas Powder Co. v. E.I. duPOnt De Nemours*, 224 USPQ 409 (Fed. Cir. 1984). With respect to claims 1-9, 11 19 and 20-21, an artisan would plainly not have to engage in undue experimentation because there are numerous replication initiating sequences that are described in the Specification or are known in the art, which function in fungi, including, for example, ANSI and AMA sequences of *Aspergillus nidulans*. Thus, undue experimentation is not required to practice claims 1-9, 11, 19 and 20-21.

For the foregoing reasons, Applicants submit that the claims overcome the enablement rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 1-9 and 11-21 under 35 U.S.C. 112, second paragraph

Claims 1-9 and 11-21 are rejected under 35 U.S.C. 112 as allegedly indefinite on a number of grounds, as follows:

A. The Examiner contends that it is not clear whether the population of DNA vectors contains more than one variant of the polynucleotide sequence or whether the polynucleotide sequence is a single DNA vector that contains different variations, or a single variation DNA in each vector.

By its plain meaning, the phrase "wherein the population of DNA vectors contains more than one variant of the polynucleotide sequence" clearly sets forth that the population of DNA vectors carries different polynucelotides sequences. *See also* the specification at page 5. In this regard, although one vector in the population may be identical to another vector in the population and a single vector in the population may contain two different versions of the polynucleotide sequence of interest, there are at least some vectors in the population that vary from other vector in the population in that they carry different versions of the polynucleotide sequence of interest. This definition is also consistent with the recitation of "a library of polynucleotide sequences of interest," as recited in claim 1 and as used through out the specification. However, as amended, claim 1 now further clarifies that "there are vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest." Reconsideration and withdrawal of the rejection is therefore respectfully requested.

B. The Examiner also contends that the recitation "selection pressure" is unclear, and requests clarification of what applicant means by this recitation. The phrase "selection pressure" is defined in the specification, *inter alia*, on page 9 to 10, as culturing the fungal cells containing the DNA vectors in the presence or absence of an appropriate selective agent that permits the selection or screening of cells containing the selection marker from cells which do not contain the selection marker. Numerous examples are also provided of suitable selection pressures, including a biocide and toxicity to heavy metal. In this regard, "selection pressure" clearly refers to a pressure placed on a cell (such as, a growth condition, temperature, a physical condition, or a chemical condition), which permits the selection or screening of the cells due to their either having or not having the selection marker. The term selection pressure is also a term that it well understood in the art, and which has been used in many patent and not-patent publications for some time. Therefore, the recitation "selection pressure" is clearly not indefinite. Reconsideration and withdrawal is therefore respectfully request.

C. The Examiner contends that the recitation of "polynucleotide sequence" in step (i) is confusing since step (ii) also recites "polynucleotide sequence," and Applicant is requested to clarify. As amended, the claims now recite "a fungal selection marker polynucleotide sequence," "a fungal replication initiating polynucleotide sequence," and "a polynucleotide sequence of interest." The

use of the additional descriptive terms in combination with the term polynucleotide sequence clearly distinguishes one sequence from another in the claims. Applicant respectfully request reconsideration and withdrawal of the rejection.

D. The Examiner states that claim 1 recites "variant of the polynucleotide," and alleges that this recitation is vague and indefinite. The Examiner further alleges that the specification does not have a definition for "variants" and it is not clear which variants are included.

As used in the claims, and as previously discussed, the term "variants" refers to variation present in DNA sequences by virtue of different DNA sequence of interest being carried by different vectors in the population of vectors, which are then used to transform fungal cells, and which fungal cells are then subject to the selection and screening following a selection pressure. Thus, the term "variants" is used in the claims simply to indicate that the polynucleotides carried by the vectors are not all the same, that is, there are different versions of the polynucleotide sequence of interest. Similarly, the specification (at pages 5-6) defines "variants" as including, *inter alia*, polynucleotide sequences that differ from a parent nucleotide sequence of interest and were prepared by mutagenesis techniques, polynucleotide sequences that are natural allelic variations of a parent polynucleotide sequence, and polynucleotide sequences that are obtained from different sources and encode different, but related polypeptides (i.e., related by activity or function). Nevertheless, in order to respond to the Examiner's concern, the term "variants" has been replaced by the phrase "different versions of the polynucleotide sequence of interest."

Regarding the Examiner's assertions that it is not clear which variants are included, this assertion is not applicable to the claimed methods because all variants are included. That is, the claimed methods are not restricted in any manner to screening and selecting a certain class of variant polynucleotides. In this regard, the specification is clear that all variants are included. See the specification at page 5 to 6. Reconsideration and withdrawal of the rejection are respectfully requested.

E. The Examiner contends that the recitation "desired characteristic" is not clear and is not defined in the specification. The recitation "desired characteristic" is clearly defined in the specification on pages 23 to 24, as broadly including any activity or function which is apparent following the cultivation of the cells under a selection pressure. The specification (at page 23-24) also provides numerous examples of what a desired characteristic is, as follows:

Thus, if the polynucleotide sequence of interest encodes a polypeptide with a

certain activity or function the selection will only allow the transformants expressing a polypeptide with the desired activity or function, to grow. Thus, if the polynucleotide sequence of interest encodes a polypeptide with a certain activity or function, the screening will be performed to identify transformants expressing a polypeptide with the desired activity or function. For instance, if the polynucleotide sequence of interest encodes an enzyme, such as a lipase, the selection or screening step c) will be performed to identify transformants expressing lipase activity. If it is desired that the lipase to be identified as a specific characteristic, such as a high thermostability, the screening is to be performed under conditions (typically temperatures) at which lipases with the desired high thermostability can be identified.

Analogously, if the polynucleotide sequence of interest is a control sequence such as a promoter sequence the selection or screening step c) is performed under condition in which promoter activity can be assessed. Typically, in the library the promoter polynucleotide sequences of interest are operably linked to a second sequence to be transcribed (e.g. a polypeptide encoding sequence) so that the promoter activity can be assayed with reference to the transcription of said second sequence.

Therefore, contrary to the rejection, the term "desired characteristic" is both clearly defined and clearly exemplified in the specification. Reconsideration and withdrawal of the rejection are respectfully requested.

F. The Examiner alleges that the recitation in claim 3 of "wherein the polynucleotide sequence encodes a polypeptide or is a control sequence; or wherein the polynucleotide sequence encodes a polypeptide or part thereof and further comprises a control sequence involved in the expression of the polypeptide or a part of such control sequence" is indefinite because it is not clear which polynucleotide sequence encodes which polypeptide.

As amended, claim 3 now recites that the vector further comprises a control sequence involved in the expression the polypeptide of interest. New claim 30 recites wherein the polypeptide of interest comprises a control sequence. Support for amended claim 3 and new claim 30 is found in the specification as originally filed on page 8, lines 3-18, and in original claim 3. Applicant submits that the claims, as amended, overcome the indefiniteness rejection.

G. The Examiner also contends that the phrase "a portion thereof" in claim 4 is indefinite. Applicant respectfully submits that the phrase "a portion thereof" is used in its plain and ordinary meaning, and thus, is clearly understood in the context of claim 4 to

refer to a polynucleotide sequence that is less than all of the polynucleotide sequence encoding a receptor or an antibody. Applicant therefore respectfully requests reconsideration and withdrawal of the rejection.

H. The Examiner states that the recitation "the control sequence" in line 1 of claim 7 lacks proper antecedent basis. Applicant submits that the antecedent basis problem has been corrected be making claim 7 depend from claim 3 instead of claim 1.

I. The Examiner states the recitation "the selective marker" in lines 1-2 of claim 9, lacks proper antecedent basis. In claim 9, the recitation "the selective marker" has been changed to "the selection marker polynucleotide sequence" to provide proper antecedent basis.

J. The Examiner states that the recitation "genes which encode a product which provides for resistance to biocide or viral toxicity..." needs clarification. As suggested by the Examiner, claim 9 has been amended to recite that the group of genes encode a product which is responsible for one of the following: resistance to biocide or viral toxicity, resistance to heavy metal toxicity, prototrophy to auxotrophs.

K. The Examiner also contends that claim 9 recites an improper Markush group. Claim 9 has been amended in the manner proposed by the Examiner.

L. The Examiner contends that claims 12-16 are indefinite by recitation of % identity because sequence identity between two sequences does not have a common meaning within the art. Claims 12 and 13 are amended to specify that the sequence identity is determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3. Applicant respectfully requests reconsideration and withdrawal of the rejection.

M. Claim has been rejected as indefinite for the recitation "a respective functional subsequence thereof." Applicant respectfully submits that this rejection is rendered moot.

N. Claim 18 has been rejected as indefinite on the basis that the recitation "wherein the modification of parent polynucleotide sequence" lacks antecedent basis. This rejection is rendered moot by the amendment to claim 18.

O. Claim 18 has been rejected as indefinite for the recitation "preferably." This rejection is rendered moot by the amendment to claim 18.

IV. The Rejection of Claims 1-9 and 18-21 under 35 U.S.C. 102

Claims 1-9 and 18-21 stand rejected under 35 U.S.C. 102 as being anticipated by Christensen, WO 98/01470 for the reasons set forth in paragraph 16 of the Office Action. This rejection is respectfully traversed.

The present invention is directed to methods for constructing and selecting or screening a library of polynucleotide sequences of interest in filamentous fungal cells by (a) transforming fungal cells with a population of DNA vectors having a fungal selection marker polynucleotide sequence, a fungal replication initiating sequence polynucleotide sequence and a polynucleotide sequence of interest, (b) cultivating the cells under a selection pressure, (c) selecting or screening for one or more transformants expressing a desired characteristic; and (d) isolating the transformant(s) of interest.

Christensen clearly does teach or even suggest this method. Christensen discloses a transcription factor and various homologous transcription factors, which regulate the expression of the alpha-amylase promotor in filamentous fungi, and therefore can be used to produce a polypeptide of interest in a host. Although Christensen teaches transforming a fungal cell with a vector carrying this transcription factor, including on an autonomous replicating vector, Christensen plainly does not teach transforming a cell with a population of DNA vectors having a fungal selection marker polynucleotide sequence, a fungal replication initiating sequence polynucleotide sequence and a polynucleotide sequence of interest, applying a selection pressure, and selecting or screening for one or more transformants expressing a desired characteristic. In fact, Christensen makes no mention, or even suggestion, of the combination of a fungal replication initiating sequence and a selective marker, not to mention the use of this combination in the methods claimed by Applicant. Indeed, the Examiner's summary of Christensen clearly fails to set forth a proper anticipation rejection, as the Examiner does not point out where each and every element of claim 1 is disclosed. Thus, Christensen clearly does anticipate the present invention.

Accordingly, Applicant submits that the claims overcome this rejection under 35 U.S.C. 102. Applicant respectfully request reconsideration and withdrawal of the rejection.

V. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: September 4, 2001

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jesper Vind Serial No.: 09/426,038

Confirmation No: 1334 Group Art Unit: 1627

Filed: October 25, 1999 Examiner: P. Ponnaluri

For: Methods Of Constructing And Screening A DNA Library Of Interest In Filamentous Fungal Cells

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE SPECIFICATION:

The paragraph on page 1, line 27 to page 2, line 3 has been amended as follows:

Often, however, a polynucleotide sequence identified by screening in yeast or bacteria cannot be expressed or is expressed at low levels when transformed into production relevant filamentous fungal cells. This may be due to any number of reasons, including differences in codon usage, regulation of mRNA levels, translocation apparatus, post-translational modification machinery (e.g., cysteine bridges, glycosylation and acylation patterns), etc.-

The paragraph on page 49, line 27 to page 50, line 2 has been amended as follows:

--The *amdS* gene flanked by the 230 bp repeated sequences was obtained from pJRoy47 as a *Swa*I/*Pme*I fragment and inserted into *Eco*RV/*Stu*I digested pDM156.2 to create pDM222.A (Figure 3). pDM222.A was digested with *Eco*RI and the 4.4 kb *Eco*RI fragment containing the *pyrG* deletion cassette was gel purified using QIAQUICK [Qiaquick] Gel Extraction Kit (Qiagen, Chatsworth, CA) prior to transformation.--

The paragraph on page 50, lines 3-12, has been amended as follows:

Spores of *Fusarium venenatum* MLY3 were generated by inoculating a flask containing 500 ml of RA sporulation medium with three $1cm^2$ mycelia plugs from a minimal medium plate and incubating at 28°C, 150 rpm for 2 to 3 days. Spores were harvested through MIRACLOTH [Miracloth] (Calbiochem, San Diego, CA) and centrifuged 20 minutes at 7000 rpm in a SORVALL [Sorvall] RC-5B centrifuge (E. I. DuPont De Nemours and Co., Wilmington, DE). Pelleted spores were washed twice with sterile distilled water, resuspended in a small volume of water, and then counted using a hemocytometer.

IN THE CLAIMS:

1. (Amended.) A method of constructing and selecting or screening a library of polynucleotide sequences of interest in filamentous fungal cells, wherein the method comprises:

(a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises [a polynucleotide sequence encoding]:

(iii) [a polynucleotide sequence encoding] a fungal selection marker polynucleotide sequence and a fungal replication initiating polynucleotide sequence, wherein the marker and the replication initiating sequence do not vary within the population; and

(iv) a polynucleotide sequence of interest, wherein there are vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest [wherein the population of DNA vectors contains more than one variant of the polynucleotide sequence];

(b) cultivating the cells under a selection pressure;

(c) selecting or screening for one or more transformants expressing a desired characteristic; and

(d) isolating the transformant(s) of interest.

2. (Unchanged.) The method according to claim 1, wherein the library of polynucleotide sequences of interest is prepared by random mutagenesis or naturally occurring allelic variations of at least one parent polynucleotide sequence having or encoding a biological

activity or function of interest.

3. (Amended) The method of claim 1, wherein the polynucleotide sequence of interest further comprises a control sequence [encodes a polypeptide or is a control sequence; or wherein the polynucleotide sequence encodes a polypeptide or part thereof and further comprises a control sequence involved in the expression of the polypeptide or a part of such control sequence].

4. (Amended.) The method according to claim [3]1, wherein the [polypeptide is] polynucleotide sequence of interest encodes a hormone, an enzyme, a receptor or a portion thereof, an antibody or a portion thereof, or a reporter, or a regulatory protein.

5. (Unchanged.) The method of claim 4, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, or a ligase.

6. (Unchanged.) The method according to claim 4, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, a proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

7. (Amended) The method according to claim [1] 3, wherein the control sequence is an enhancer sequence, a leader sequence, a polyadenylation sequence, a propeptide sequence, a promoter, a replication initiation sequence, a signal sequence, a transcriptional terminator or a translational terminator.

8. (Unchanged.) The method of claim 7, wherein the promoter is derived from the gene encoding *Aspergillus oryzae* TAKA amylase, NA2-tpi and *Aspergillus niger* or *Aspergillus awamori* glucoamylase.

9. (Amended) The method according to claim 1, wherein the [selective marker] selection marker

polynucleotide sequence is selected from the group of genes which encode a product which [provides] is responsible for one of the following: resistance to biocide or viral toxicity, resistance to heavy metal toxicity, [or] prototrophy to auxotrophs.

11. (Amended.) The method of claim 9, wherein the [selective marker] selection marker polynucleotide seqeunce is a gene selected from the group consisting of *argB* (ornithine carbamoyltransferase), *amdS* (acetamidase), *bar* (phos-hinothricin acetyltransferase), *hemA* (5-aminolevulinate synthase), *hemB* (porphobilinogen synthase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *prn* (proline permease), *pyrG* (orotidine-5'-phosphate decarboxylase), *pyroA*, *riboB*, *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase).

12. (Amended) The method of claim 1, wherein the replication initiating polynucleotide sequence is a nucleic acid sequence selected from the group consisting of:
    (a) a replication initiating sequence having at least [50]80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3, and is capable of initiating replication; and
    (b) a replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x SSC, 0.2% SDS[; and
    (c) a subsequence of (a) or (b), wherein the subsequence has replication initiating activity].

13. (Amended) The method of claim 12, wherein the [nucleic acid] replication initiating polynucleotide sequence has at least [50]80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

14. (Amended.) The method of claim 12, wherein the replication initiating polynucleotide sequence is obtained from a filamentous fungal cell.

15. (Unchanged.) The method of claim 14, wherein the filamentous fungal cell is a strain of *Aspergillus*.

16. (Unchanged.) The method of claim 15, wherein the strain of *Aspergillus* is obtained from a strain of *A. nidulans*.

17. (Twice amended.) The method of claim 12, wherein the replication initiating polynucleotide sequence has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2[, or is a respective functional subsequence thereof].

18. (Amended.) The method of claim 2, wherein the [modification of the parent] polynucleotide sequence of interest [is performed] was created by mutagenesis, [preferably] by random mutagenesis, by use of a physical or chemical mutagenizing agent, by use of a doped oligonucleotide, by DNA shuffling, [or] by subjecting the nucleic acid sequence to PCR generated mutagenesis, or by use of any combination thereof.

19. (Unchanged.) The method of claim 18, wherein the polynucleotide sequences of interest are obtained by in vivo recombination between two or more homologous nucleic acid sequences encoding a polypeptide or a regulatory sequence, or any combination of both, comprising:
    (a) identifying at least one conserved region between the polynucleotide sequences of interest;
    (b) generating fragments of each of the polynucleotide sequences of interest, wherein said fragments comprise the conserved region(s) of (a); and
    (c) recombining the fragments of (b) by using the conserved region(s) as (a) homologous linking point(s).

20. (Unchanged.) The method according to claim 1, wherein the filamentous fungal cell transformed with the population of DNA vectors is a cell of a strain of *Acremonium*,

Aspergillus, *Coprinus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliopthora*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium* or *Trichoderma*.

21. (Unchanged.) The method according to claim 20, wherein the cell is an *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus nidulans*, *Coprinus cinereus*, *Fusarium oxysporum*, or *Trichoderma reesei* cell.